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Formulation of concentrated oil-in-water-in-oil double emulsions for fragrance encapsulation

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Abstract

We report the formulation of a concentrated double oil-in-water-in-oil (O/W/O) emulsion for fragrance encapsulation. As their water-in-oil-in-water homologous, these O/W/O emulsions, seldom described in literature, also require the use of two antagonist surfactants: a hydrophilic one (HS) to stabilize the fragrance-in-water droplets and a lipophilic one (LS) to stabilize the aqueous globules dispersed in oil, containing themselves the direct emulsion. An important issue in cosmetics is the necessity to adapt the formulation to each fragrance, so that a major progress would be the successful elaboration of an O/W/O emulsion insensitive to fragrance changes. In order to approach such a composition, two model fragrances composed of 10 and 13 molecules representative of the most used molecules in this domain were specially assembled for the study. The aim was to identify possible hydrophilic and lipophilic stabilizers

leading to a double O/W/O emulsions and determine compositions able to encapsulate both fragrances. The double emulsion was prepared by a two-step process, allowing varying the double emulsion composition *i.e* the amount of both surfactants as well as the quantity of droplets inside the globules. By plotting “feasibility diagrams” we can propose a best composition shared by the two fragrances. Such a double emulsion is composed of 20 wt% of fragrance droplets with respect to the globule volume, 75 wt% of globules with respect to the total emulsion, 7.5 wt% of HS with respect to the intermediate aqueous phase and 10 wt% of LS with respect to external oil phase. The robustness of this optimized composition against molecule changes was tested using a third fragrance. Then the encapsulated efficiency was measured showing the high encapsulation rate (close to 99%) of the selected system and a gas chromatography analysis of each molecule composing the complex fragrance showed no alteration of the encapsulated fragrance.

Keywords: emulsions, O/W/O emulsion, fragrance, encapsulation, formulation

1. Introduction

Among multiple emulsions [1-7], double oil-in-water-in-oil emulsions are dispersions consisting of oil-in-water emulsions themselves dispersed as drops in an oil outer phase. Owing to their compartmentalized structure, they are interesting colloidal systems for encapsulating either aqueous or lipophilic or both kinds of species [8-19]. Such systems may be produced in a one-step process using one surfactant [10,11,20-23]. Although simple, this process does not offer sufficient versatility and does not allow the control of the globule size. Moreover, the inner and the outer oils are necessarily the same, which is not relevant for encapsulation. This is why we decided to use two-step process [8,9,15,16,18,19,24-27] very common for W/O/W double emulsions. For W/O/W emulsions, first, water containing the hydrophilic drug (or the

encapsulated hydrophilic species) is dispersed as small droplets in the oil containing a preferably lipophilic stabilizer, then, this reverse emulsion is dispersed in water containing a hydrophilic stabilizer [8,9]. As a consequence of the simultaneous presence of a direct (oil-in-water) and a reverse (water-in-oil) emulsion, two kinds of stabilizers are required. In order to improve the stability of the water-in-oil emulsion, a water-soluble species like salt or glucose is added in the innermost aqueous phase. An osmotic regulator is also added in the outermost aqueous phase [28]. Due to the numerous constituents, these systems are often considered complex and their use as capsules are limited despite their large loading ability. Another difficulty comes from their preparation method: during the second emulsification step, fragmentation must be controlled in order to avoid destruction of the first emulsion or a connection of the two (innermost and outer) compartments that would lead to a dilution and immediate release of the encapsulated drug [9]. Also, their sensitivity towards the encapsulated species is often a brake to their use. For a recent review about double emulsions in the domain of food, the reader can refer to [29]. Indeed, this paper is dedicated to a critical evaluation of the emulsification preparation methods, the range of encapsulated components and emulsion composition, the balancing of the osmotic pressure, the stabilization by increased viscosity or gelation...in the food domain considering both W/O/W and O/W/O double emulsions. Among the literature on double emulsions, the majority concerns W/O/W emulsions while O/W/O are more scarce [19-21,24,25,30-33], and are usually devoted to food [19,24,25,31-33]. The most common HS in food are the Tween 20 surfactant [26,30] and the Sodium Caseinate protein [19,24,25,33] and the most common LS are Span 80 as for example in [32] and polyglycerol polyricinoleate (PGPR) [19,24-26,30,33]. Kinetic stability is often enhanced using gelation of the water phase as for example with pectin [26,30] or at the interface. In the present case, the application domains are cosmetics, laundry and detergency. In such domains, the use of animal proteins as sodium caseinate originating from milk should be avoided. Also, in the present

study, double emulsions are only a first stage before polymerization of the intermediate aqueous phase [34] so that a kinetic stability of few hours is large enough.

Encapsulation may be achieved through very diverse processes among which one can cite the following: *i)* layer-by-layer [35] deposition of alternate layers of polyelectrolytes of opposite charges onto a sacrificial particle that has to be removed. The fragrance filling occurs after the capsule preparation, *ii)* spray drying [36] of an emulsion that solidifies the drops during drying *iii)* Spray-coating [37] allowing encapsulating continuously solid particles. Particles are fluidized and a coater is pulverized onto the particles. The coater is then solidified by decreasing the temperature or by solvent evaporation, *iv)* solvent evaporation [38]. Usually the organic phase containing a volatile solvent, a polymer and the species to be encapsulated is emulsified in a water phase in which the polymer is not soluble. Stirring is maintained until the volatile solvent has diffused through the aqueous phase and has evaporated while the polymer has precipitated. The obtained capsules are usually very porous, *v)* coacervation [39] is based on the separation between a polymer-rich and a polymer-poor phase provoked by the polymer desolvation induced by temperature, pH... A cross-linker may be added to rigidify the shell, *vi)* colloidosomes [40] are obtained through the sintering of particles adsorbed at the interface of a so called Pickering emulsion, that is to say a particle stabilized emulsion, *vii)* interfacial polycondensation, widely used, is based on the formation of an emulsion containing a hydrophobic monomer in its dispersed lipophilic phase and a hydrophilic monomer in its continuous aqueous phase. The two monomers can react at the interface forming a primary membrane that thickens during polymerization [41,42], *viii)* suspension polymerization [43,44]. The monomer, the initiator and the species to be encapsulated are solubilized in the oily dispersed phase of a direct emulsion containing the stabilizer. Polymerization is initiated and occurs in the oily phase. As the chain length increases, the polymer becomes less soluble in the oily phase and precipitates at the interface forming a shell, *ix)* emulsion polymerization is one

of the most used encapsulation technique. A lipophilic monomer is progressively added in an aqueous solution containing surfactant, at a concentration that can be chosen below its critical micellar concentration and an initiator. This leads to the formation, under stirring, of monomer drops stabilized by the surfactant. After polymerization initiation, the oligomers in formation become water insoluble and migrate into the monomer drops in which nucleation occurred. The species to be encapsulated may be directly added with the monomer or added after so that it diffuses into the capsules.

In the present work, we wish to elaborate fragrance capsules in which the fragrance is not soluble in the polymerization locus. This is the reason why we chose in a first step to formulate fragrance-in-water-in-perfumery oil emulsions in order to, later, polymerize the aqueous intermediate phase. In this context, the aim of the present paper is the formulation of a fragrance-in-water-in-oil double emulsion able to be used for different fragrances. Encapsulation of fragrance is a widely spread first step in many applications like softeners, deodorants and so on. The studied fragrances were model systems composed of a variety of molecules with different functional groups that are commonly employed in perfumery. Rather than just diluting the lipophilic fragrance into an oily perfumery solvent, the proposed strategy is the encapsulation of the fragrance in an emulsion. The choice of an O/W/O is imposed by the polarities of the fragrance and the solvent. The advantage of these multiple emulsions is the presence of the intermediate water phase to slow down the leakage of the fragrance. In order to improve the stability of the water-in-oil emulsion, a water soluble species like salt or glucose is added in the intermediate aqueous phase [26]. O/W/O systems are very rare in the literature compared to W/O/W emulsions. As described above, the two-step process has been chosen and the use of a couple of antagonist surfactants is required. Therefore, a hydrophilic one has been selected to stabilize the direct emulsion and a lipophilic one to stabilize the inverted emulsion containing the direct emulsion. To determine a possible composition, we adopted a progressive

strategy by first seeking surfactants able to stabilize concentrated simple emulsions (direct and reverse), authorized in cosmetics and not dependent on pH to avoid restriction of possible uses. Usually, the choice of the surfactant to stabilize an emulsion with almost equal volumes of both phases is based on its Hydrophilic Lipophilic Balance (HLB) [45] given by the provider. However, in the present case, this is only an indication, since we targeted concentrated emulsions with a much larger amount of dispersed phase compared to the continuous one (*i.e.* 90 wt% for the direct emulsion and 75 wt% for the reverse emulsion). Such high dispersed phase volume fractions were chosen to reach larger loadings of the capsules and a better control over the drop sizes [46]. Therefore, we swept different available surfactants and tested their ability to stabilize the simple concentrated emulsions. In a second part of the present paper, we report the formulation of the double emulsions where we varied the compositions in dispersed phase and hydrophilic surfactant (in the intermediate phase) concentrations for the two model fragrances. This led us to plot stability or feasibility diagrams, based on macroscopic and optical microscopy observations of the systems. From these diagrams, we selected a composition common to the two fragrances and tested its robustness against a third fragrance. Finally, the encapsulation rate of the selected system of double emulsion was assessed using gas chromatography.

2. Materials and Methods

2.1. Chemicals

All the reagents were used without further purification. The tested lipophilic surfactants (LS) were polyglycerol polyricinoleate (PGPR) with a molar mass of 3000 g.mol⁻¹ (HLB = 1.5) graciously provided by Paalsgard and Span80 (molar mass around 420 g.mol⁻¹, HLB = 4.3) purchased from Sigma-Aldrich. The selected hydrophilic surfactants (HS) were Tween 60 (molar mass around 1310 g.mol⁻¹, HLB = 14.9), Tween 20 (molar mass around 1220 g.mol⁻¹,

HLB = 16%) and Tergitol 15-S-12 (molar mass around 760 g.mol⁻¹ and HLB = 14.5). All these HS were purchased from Sigma-Aldrich. The external oil was isopropyl myristate (IPM, density equal to 0.850 g.mL⁻¹) chosen because it is widely used in perfumery. It was provided by Takasago Europe Perfumery Laboratory (T.E.P.L). Two different fragrances, hereafter named A and B and composed of 10 and 13 molecules respectively, have been specially assembled by T.E.P.L. Their composition is detailed in Tables 1 and 2 and their main global characteristics are summed up in Table 3. Sodium Chloride (NaCl, molar mass 58.44 g.mol⁻¹), incorporated in the water phase to increase the reverse emulsion stability was purchased from Sigma-Aldrich. Milli-Q water was used in all emulsions preparation.

Table 1: Composition of fragrance A in wt%.

Perfumery name	Chemical name	wt %	Molecular Weight (g/mol)	LogP*	T _b ** (°C)
ALDEHYDE C12 LAURIC	Dodecanal	5	184.32	5.07	257
TH LINALOOL	3,7-dimethyloctan-3-ol	15	158.28	3.52	155
HEDIONE	methyl 2-(3-oxo-2-pentylcyclopentyl)acetate	20	226.32	2.91	110 (0.2 mmHg)
METHYL HEXYL KETONE	2-Octanone	10	128.22	2.44	173
BOISAMBRENE	Ethoxymethyl-cyclododecyl ether	5	242.40	5.48	94 (1 mmHg)
DIMETHYL ANTHRANILATE	Methyl N-methylanthranilate	5	165,19	2.66	256
DECALACTONE	5-Hexyloxolan-2-one	10	170,25	2.36	270
CLONAL	Dodecane nitrile	5	181.32	4.90	277
EUCALYPTOL	1,3,3-triméthyl-2-oxabicyclo[2,2,2]octane	5	154.25	2.91	176
ISO BORNYL ACETATE	1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate	20	196.29	4.04	225

* LogP corresponds to the relative solubility in water and octanol and is defined by Eq. 1

** T_b is the boiling temperature taken at 760 mmHg, otherwise specified.

Table 2: Composition of fragrance B in wt%.

Perfumery name	Chemical name	wt %	Molecular Weight (g/mol)	LogP	T _b (°C)
HEXENYL ACETATE CIS 3	cis-3-Hexenyl acetate	5	142.20	2.34	167
TRIPLAL	2,4-dimethylcyclohex-3-ene-1-carbaldehyde	5	138.21	2.36	196
EUGENOL	2-methoxy-4-(2-propenyl)-phenol	5	164.20	2.40	254
CYCLACET	Tricyclododecenyl Acetate	10	192.26	2.88	295
CITRAL	3,7-dimethylocta-2,6-dienal	5	152.24	2.95	229
CITRONELLOL 950	3,7-Dimethyloct-6-en-1-ol	5	156.27	3.25	225
ALLYL HEPTANOATE	prop-2-enyl heptanoate	5	170.25	3.60	210
DAMASCONE DELTA	1-(2,6,6-trimethyl-1-cyclohex-3-enyl)but-2-en-1-one	2	192.30	3.62	256
IONONE BETA	4-(2,6,6-trimethyl-1-cyclohexene-1-yl)-3-butene-2-one	5	192.30	3.71	259.5
ALDEHYDE C11 UNDECYLENIC	10-undecenal	5	168.28	4.05	223
VERDOX	(2-tert-butylcyclohexyl) acetate	38	198.31	4.06	221
ISORALDEINE 70	1-(2,6,6-trimethyl-2-cyclohexen-1-yl)penten-3-one	5	206.33	4.23	285
HEXYL CINN ALD	2-Benzylideneoctanal	5	216.32	5.00	318

LogP, widely used in perfumery and in pharmacy to describe the molecules' polarity, is defined as:

$$\text{LogP} = \text{Log}\left(\frac{C_{\text{octanol}}}{C_{\text{water}}}\right) \quad (1)$$

where P is the partition coefficient, C_{octanol} and C_{water} the respective concentrations of the species in octanol and water. Log P is an indication of the relative solubility of the molecule in water and in octanol. Indeed, a positive and high value of LogP means that the considered species is more soluble in octanol than in water or, in other words, it means that the species is lipophilic. Inversely, a negative LogP value is obtained for a hydrophilic species while LogP equals to zero means that the molecule is equally soluble in water and in octanol. Perfumes are mixtures of a large number of molecules, it is assumed that LogP of a mixture is given, with a good approximation [47], by the molar fraction weighted average value. For both fragrances A and

B, the values are positive meaning they are more soluble in octanol than in water. It is also worth noticing that the fragrances have close LogP values (see Table 3).

Table 3: Properties of both fragrances.

	Fragrance A	Fragrance B
LogP	3.38	3.52
Refractive index	1.454	1.475
Density (g/cm ³)	0.869	0.887
Interfacial tension against water (mN.m ⁻¹)	21.8	34.6

2.2 Emulsion size characterization

The emulsions size distributions were measured using static light scattering by means of a Malvern Mastersizer MS2000 granulometer.

For the simple emulsion, the measured scattered intensity as a function of the angle was transformed into the size distribution using the Mie theory. Reverse emulsions were diluted with IPM prior to measurement. The refractive index of the dispersed water phase was taken equal to 1.333 as no significant difference has been detected for the brine (0.1 M NaCl) compared to pure water. The refractive index of the continuous IPM oil was taken equal to 1.434. For the direct emulsion, the refractive indices of fragrances A and B were 1.454 and 1.475 respectively. All the refractive indices have been measured using an Abbé refractometer.

For the multiple emulsions, as oily globules were themselves composed of water droplets, they were not optically homogeneous; we then considered that the Mie theory was not adapted. To avoid this difficulty, we chose to use the simplified Fraunhofer theory where the principles of geometrical optics apply and the drops are approximated by opaque discs. This approximation is usually valid for drops larger than, or of the order of, 10 μm . However Nollet *et al.* [9] showed that it can also be applied down to 5 μm

with a correct determination of the mean drop size even if the drops size distribution is misestimated.

In all cases, we checked by optical microscopy that the drop size was not altered by the dilution required for static light scattering measurements.

For all types of emulsions, the volume-average diameter $D[4,3]$ and the polydispersity index U were determined, they are defined as following:

$$D[4,3] = \frac{\sum_i N_i D_i^4}{\sum_i N_i D_i^3} \quad U = \frac{1}{D_m} \frac{\sum_i N_i D_i^3 |D_m - D_i|}{\sum_i N_i D_i^3} \quad (2)$$

where N_i is the total number of droplets with diameter D_i . D_m is the median diameter, *i.e.*, the diameter for which the cumulative undersized volume fraction is equal to 50%. The polydispersity index U is therefore the volume average difference with the median diameter normalized by the median diameter. It is an indicator of the drops size distribution width. Both $D[4,3]$ and U were directly given by the Malvern Mastersizer software.

2.3. Emulsion preparation

2.3.1. Simple direct emulsion

All the direct emulsions were prepared following the same procedure. First, a concentrated polydisperse emulsion was obtained by manually incorporating the chosen A or B fragrance (90 wt% with respect to total emulsion weight) into an aqueous phase composed of salted water (0.1 M of NaCl) and HS at 30 wt% with respect to the water phase (in other words, for 100 g of a direct emulsion, the composition is the following: 90 g of fragrance dispersed in an aqueous phase composed of 0.04 g of NaCl, 3 g of HS and 6.96 g of H₂O). This crude premixed emulsion was then sheared in a Couette cell (commercialized by Ademtech) composed of two concentric cylinders spaced by a 100 μm gap.

The inner cylinder of radius $R_i = 20$ mm rotated with a speed of 680 rpm while the outer cylinder was static, leading to an applied shear rate of $14\,280\text{ s}^{-1}$. If the conditions are fulfilled for a Rayleigh instability to develop, quasi-monodisperse emulsions can be obtained [22]. An average droplets size close to $1\text{ }\mu\text{m}$ was aimed at, in order to be encapsulated in a second step. In the following, the drops of the direct emulsion will be named droplets.

2.3.2. Simple reverse emulsions

All the inverted emulsions were prepared following the same procedure. First, a polydisperse emulsion was obtained by manually incorporating 75 wt% of 0.1 M NaCl brine in IPM containing 10 wt% of LS with respect to the oil phase (for 100 g of reverse emulsion the composition was therefore as following: 75 g of aqueous solution containing 74.56 g of H_2O and 0.44 g of NaCl and 25 g of the oily phase composed of 22.5 g of IPM and 2.5 g of LS). The crude emulsion was then sheared with the same Couette cell using stators with a gap of 100 or 200 μm . The speed has been varied to determine the most appropriate one as a function of the surfactant. The drops of inverted emulsions will be named globules in the following. In order to be able to encapsulate the direct emulsion droplets, the targeted globule size was around 15 μm .

2.3.3. double emulsions

Multiple oil-in-water-in-oil emulsions were prepared following a classical two-step procedure [3,4]. First, a direct emulsion was prepared with the protocol described in the 2.3.1 section. However, in order to expand the concentration range of HS in the intermediate aqueous phase, the concentration of HS used for the stabilization of the direct emulsion was varied from 10 wt% to 30 wt% with respect to the direct emulsion aqueous phase (corresponding to 1 wt% to 3 wt% with respect to the total fragrance-in-water emulsion). This direct emulsion was sheared and the highest achievable rate ($\sim 14\,000\text{ s}^{-1}$). In a second step, the direct emulsion

described just above and composed of either fragrance A or fragrance B was diluted with salted water (0.1 M NaCl) and served as the dispersed phase of the reverse emulsion. The direct emulsion was diluted by a factor ranging from 1.5 to 18 according to the targeted fraction of inner droplets ϕ_d (corresponding to a droplets fraction varying from 5 to 60% with respect to globule volume). Moreover, the aqueous solution used for dilution could contain HS. This dilution procedure allowed controlling both the fragrance droplet fraction ϕ_d , defined as the ratio of the encapsulated droplets' volume to the globule volume, and the final hydrophilic surfactant concentration with respect to water. This diluted direct emulsion was then progressively and manually incorporated into the oil phase containing IPM and 10 wt% of LS using a spatula. The amount of incorporated direct emulsion determines the globule volume fraction ϕ_G defined as the ratio of globules' volume to total sample volume.

To sum-up the emulsions compositions are listed in Table 4 for 100 g of emulsion.

Table 4: Composition of the various emulsions. The double emulsion was obtained by incorporation into the continuous phase of the simple direct emulsion after dilution at various rates.

Simple direct O/W emulsion,	Dispersed phase: Fragrance A or B 90 g		Continuous phase: 0.04 g NaCl 3 g HS 6.96 g H ₂ O
Simple reverse W/O emulsion	Dispersed phase: 0.44 g NaCl 74.56 g H ₂ O		Continuous phase: 22.5 g IPM 2.5 g LS
Double O/W/O emulsion	Innermost phase: Fragrance A or B 3.5 to 45 g	Intermediate phase: NaCl brine 0.1M with HS at various concentrations 30 g to 71.5 g	Continuous phase: 22.5 g IPM 2.5 g LS

2.4. Double emulsion encapsulation rate determination

The encapsulation rate, defined as the amount of encapsulated species to total species ratio, was deduced from the measurement of the non-encapsulated fragrance (fragrance in IPM):

$$\varepsilon = \frac{\text{mass of encapsulated fragrance}}{\text{total mass of fragrance}} = \frac{\text{total mass of fragrance} - \text{mass of fragrance in IPM}}{\text{total mass of fragrance}} \quad (3)$$

For this aim, the emulsion was centrifuged at 1500 rpm during 25 min to induce separation of the continuous phase from the emulsion without double emulsion rupture. The continuous phase was recovered, filtrated and diluted up to 5 wt% in ethanol. This phase was analyzed with a gas chromatograph (Agilent GC 7890B) equipped with flame ionization detector (FID) coupled with Mass spectrometer 5977A (MS), a split/splitless injector (1:40 split ratio) and a data system (Enhanced ChemStation MSD Chemstation). The detector and injector temperatures were set at 250°C. A 30 m×250 µm internal diameter capillary column coated with 5%-phenyl poly(methylsiloxane) (0.250 µm film thickness) (HP-5) was used. The oven temperature was programmed from 50 °C (2 min) to 280 °C (5 min) at 10 °C/min. Two microliters of the sample were injected. Fragrance molecules are identified with MS and quantified according to an external calibration with different solutions of fragrance in ethanol on FID.

2.5. Interfacial tension measurement

The interfacial tensions were measured with the weighing drop method. This technique consisted in shaping a drop of the heaviest phase at the end of a straight needle immersed in the lighter phase in a beaker. The volume of the drop was slowly increased. When the weight of the drop became larger than the capillary forces that maintained the drop at the end of the needle, the drop detached. The beaker was placed on a precision balance. When the drop detached, its precise weight was measured, and its volume could be deduced. The operation was repeated 10 times. Just before detachment, the capillary force and the drop force are equal so that the following equation applied:

$$2\pi r f \gamma = \Delta\rho \frac{m}{\rho} g \quad (4)$$

where r is the radius of the needle ($r = 0.3\text{mm}$), f is a corrective factor reflecting the complexity of the shape of the drop when it detached, m its mass, ρ the drop density, $\Delta\rho$ the density difference between the two liquids and g the gravity acceleration taken equal to $9.81 \text{ m}\cdot\text{s}^{-2}$. The corrective f factor is given in Supporting Information S1).

3. Results and Discussion

The first step to formulate oil-in-water-in-oil double emulsions was the selection of the two antagonistic, the hydrophilic and the lipophilic, stabilizers. A first criterion was their theoretical ability to stabilize either direct emulsions (for the hydrophilic one) or reverse emulsions (for the lipophilic one) so we focused on the Hydrophilic-Lipophilic Balance (HLB) that should be lower than 7 for the lipophilic one and larger than 8 for the hydrophilic one. To avoid sensitivity toward pH, we ruled out all surfactants bearing amine, acid and other pH-sensitive groups. We also chose to privilege stabilizers inducing steric repulsions rather than electrostatic ones. A very important criterion was their acceptance in cosmetic applications (meaning that they were not harmful, carcinogenic, mutagenic or reprotoxic CMR). After this first selection, formulations of simple, direct and reverse emulsions were studied. The second step of the work consisted in formulating double emulsions. Due to the presence of the two antagonist surfactants, the formulation could be something tricky and different for each couple of hydrophilic surfactant (HS)/lipophilic surfactant (LS). Thus, we varied the quantity of the droplets inside the globules and the amount of HS (by controlled dilution of the direct emulsion previously to incorporation in the oily continuous phase) and defined the droplet volume fraction ϕ_d as the ratio of the encapsulated droplets' volume to the globule volume. The study of the direct and double emulsions was carried out for both fragrances. The resistance of the double emulsion formulation to a change in the fragrance has also been tested by using a third

fragrance named C made of 12 molecules (see Supporting Information S2 for its composition). By this progressive strategy, we think that we determined the best composition in terms of homogeneity of the globules and droplet sizes and in term of droplets encapsulation in the globules. The homogeneity of the globules and droplet sizes were appraised by optical microscopy. Finally, the encapsulation rate of the double emulsion was measured for the “optimized” system by gas chromatography.

3.1. Formulation of the direct emulsion

Among all the hydrophilic surfactants initially selected on the basis of their HLB, only three were maintained for their ability to stabilize concentrated direct emulsions with at least one of the two fragrances: Tween 60, Tween 20 and Tergitol 15-S-12. For each surfactant, a crude concentrated direct (90 wt% of dispersed phase) emulsion was obtained. Then this emulsion was sheared in the Couette cell at the maximum accessible shearing rate ($14\,280\text{ s}^{-1}$) in order to get the smallest droplet sizes. The size distribution was determined by static light scattering using Mie theory. Their characteristics, defined by Eq. 2, are reported in Table 5.

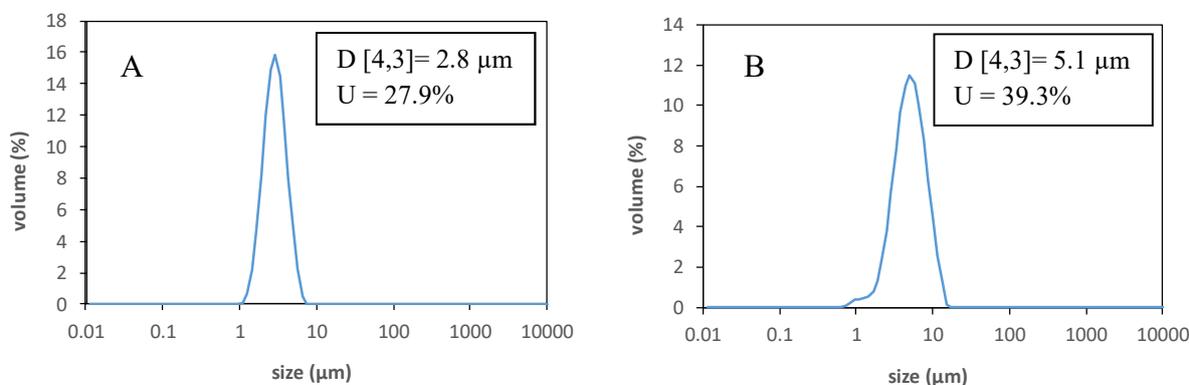
Table 5: Droplet size distribution characteristics $D[4,3]$ and U (defined by Eq 2) as a function of surfactant and fragrance

	Fragrance A		Fragrance B	
	$D [4,3]$ (μm)	U (%)	$D [4,3]$ (μm)	U (%)
Tween 20	1.8	64.4	1.1	30.9
Tween 60	unstable		1.4	28.1
Tergitol 15-S-12	0.7	28.0	0.8	28.0

The droplet size distributions measured by static light scattering are reported in Fig.1 for Tween 20 and Tergitol 15-S-12. Representative pictures obtained by optical microscopy are

reported in Supporting Information S3. Due to their small sizes, the droplets are hardly visible, especially with Tergitol 15-S-12.

a)



b)

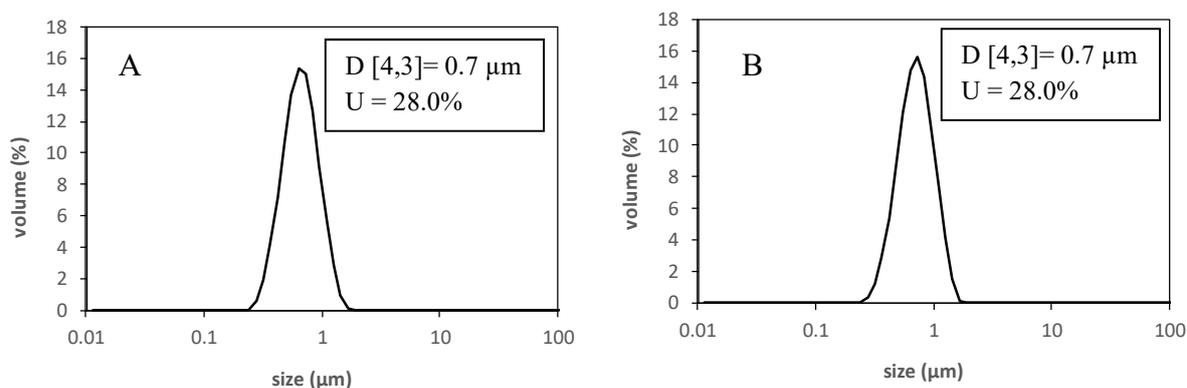


Figure 1: Drop size distributions measured by static light scattering of the direct emulsions formulated with Tween20 (top) or Tergitol 15-S-12 (bottom). The dispersed phase is fragrance A (left) and fragrance B (right). Micrographs of the corresponding emulsions are reported in Supporting Information S3.

The impossibility to formulate the direct emulsion for fragrance A with Tween 60 can be noticed. Indeed, as soon as 10 wt% of fragrance were incorporated, large and unstable drops were visible by naked eye. With the same surfactant, Tween 60, emulsions are obtained with fragrance B. As can be seen in Table 3, the interfacial tension between fragrance A and water is much lower than between fragrance B and water. As both fragrances exhibit similar LogP

values, we think that this could possibly indicate the ability of some fragrance molecules to adsorb at the interface during the emulsification of fragrance A, interfering with the adsorption of Tween 60. This phenomenon was not observed with Tween 20 that differs only by about one unit in HLB (14.9 and 16 for Tween 60 and Tween 20 respectively). As the targeted globule size is 15 μm , the required size for the direct emulsion to be encapsulated is of the order of 1 μm . As the same formula should be feasible with both fragrances, Tween 60 was not adapted to prepare double fragrance/W/O emulsions.

In order to get better insight into the understanding of the observed phenomenon, and check if our hypothesis might be correct, we carried out interfacial measurements, by the weighing drop method, between air and water in presence of the surfactant (7.5wt% well above the critical micellar concentration where it is independent of surfactant concentration). It is equal to 39.1, 43.8 and 32.6 for Tween 20, Tween 60 and Tergitol 15-S-12 respectively. Note that for emulsification one should consider the interfacial tension between fragrances and the aqueous phases so that the same experiments were carried out between fragrance B and water in presence of the same amount of surfactant. The interfacial tension is equal to 5.5, 11.3 and 2.5 for Tween 20, Tween 60 and Tergitol 15-S-12 respectively. These values can be faced to the droplets size (Fig. 2). As put into evidence by Figure 2, the resulting droplet size depends linearly on the interfacial tension.

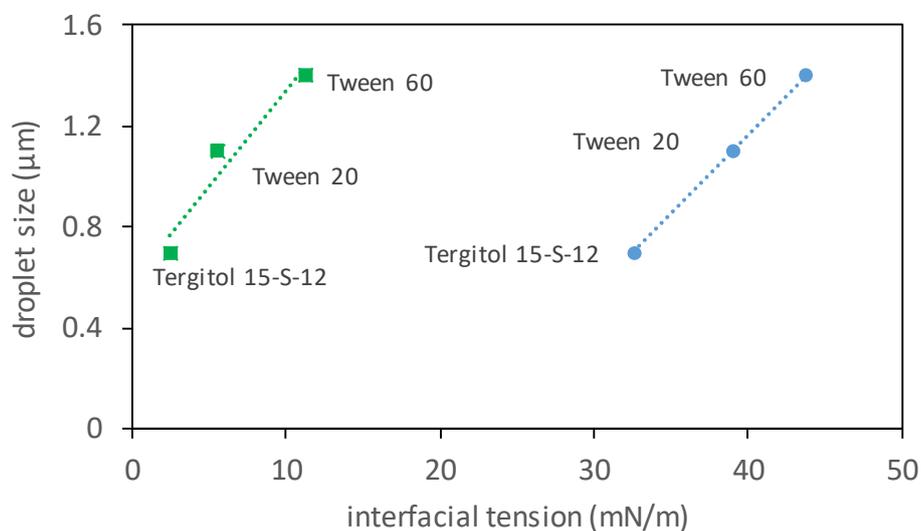


Figure 2: Obtained droplet size $D_{[4,3]}$ with fragrance B as a function of the air/water containing surfactant interfacial tension (full circles) and as a function of the fragrance B/water containing surfactant interfacial tension (full squares).

Such a plot seems indicating that, as expected, the drop size depends linearly on the interfacial tension in agreement with the definition of the critical capillary number $Ca = \frac{\sigma d}{2\gamma}$ where σ is the applied stress, d the obtained drop size and γ the interfacial tension [46]. This helps understanding the obtained sizes for the three surfactants but it does not explain the impossibility to emulsify fragrance A with Tween 60. Indeed the interfacial tension between fragrance A and an aqueous phase containing Tween 60 is equal to 8.9 mN/m to be compared with 11.3 for fragrance B. The interfacial tension for fragrance A is lower than for fragrance B as it was already the case for the fragrance/water interface (see Table 3). Nevertheless, they remain of the same order of magnitude, so that interfacial tension alone is not a determining parameter.

As a conclusion, the two surfactants Tween20 and Tergitol 15-S-12 lead to satisfactory emulsions for both fragrances so that these two surfactants have been selected for the rest of the study.

3.2. Formulation of reverse emulsion

A similar work has been done for lipophilic surfactants to stabilize the reverse brine-in-IPM emulsions. However, lipophilic surfactants (LS) are less numerous and after a first selection, we only considered two: Span 80 and PGPR. Crude emulsions were first prepared by manually incorporating the aqueous phase in the IPM oil containing 10 wt% of LS. After that, the premixed emulsion was sheared with a Couette Cell at various shear rates in order to determine the process conditions to reach 15 μm sized globules. As can be observed from Figure 3, where the globule size is plotted as a function of the shear rate for each LS, the size of the

globules decreases steeply when the shear rate is increased. The distributions become also narrower as deduced from the polydispersity index decrease. This phenomenon is similar to the one observed in direct emulsions and has been explained by the development of a Rayleigh instability under shear [46]. Example of two emulsions are given in Fig. 4. The high polydispersity at low shear rates is evidenced by the presence of smaller droplets as can be seen on both the micrograph and the drop size distribution on the left of Figures 4a and 4b. It can also be noticed that small droplets surround the larger ones as the van der Waals interaction is larger between a small drop and a large drop than between two large drops. This is commonly observed in polydisperse emulsions. It is worth noticing that from the micrograph the small drops seem very numerous while from the drop size distribution it can be concluded that they represent about 5% of the volume (Figure 4b left).

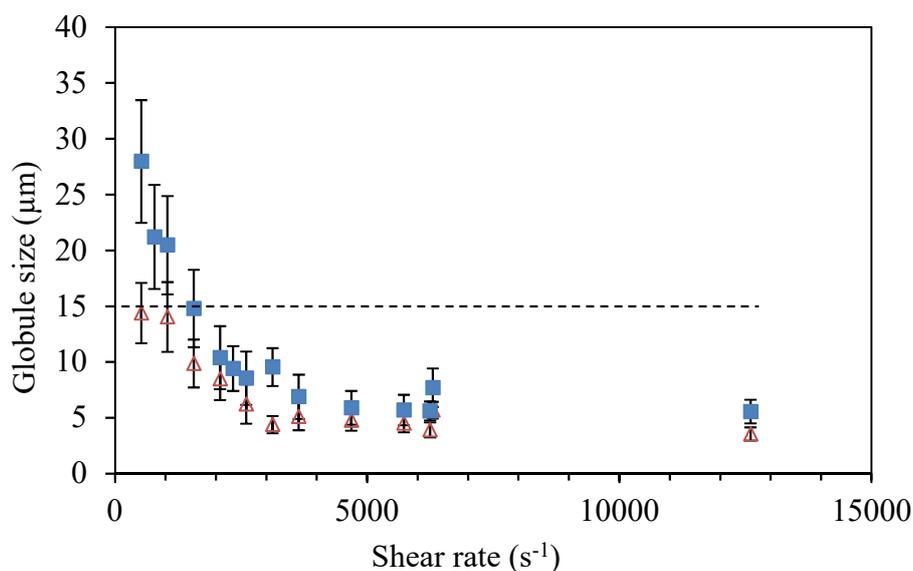
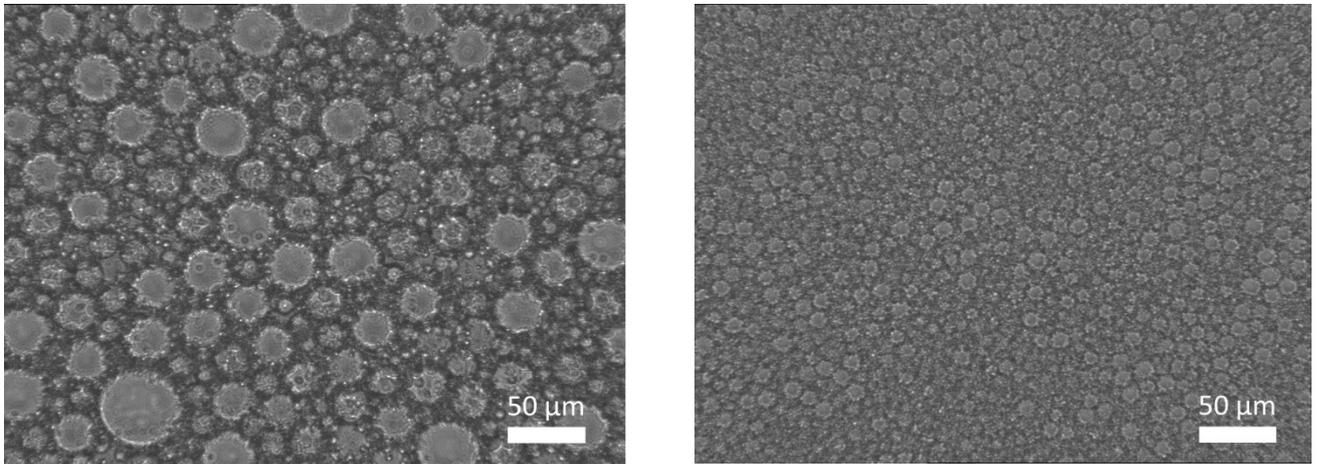


Figure 3: Evolution of the globule volume-average diameter as function of the applied shear rate for both LS: full squares PGPR and empty triangles Span 80. The error bars represent the polydispersity U of the emulsion and the targeted $15 \mu\text{m}$ size is made visible by the dashed line. Examples of drop size distributions with PGPR and Span 80 are reported in Supporting Information S4.

From Fig. 3, it can be observed that the targeted globule size of 15 μm is accessible with both stabilizers applying a shear rate of 520 s^{-1} for the Span 80 and 1560 s^{-1} for PGPR.

a)



b)

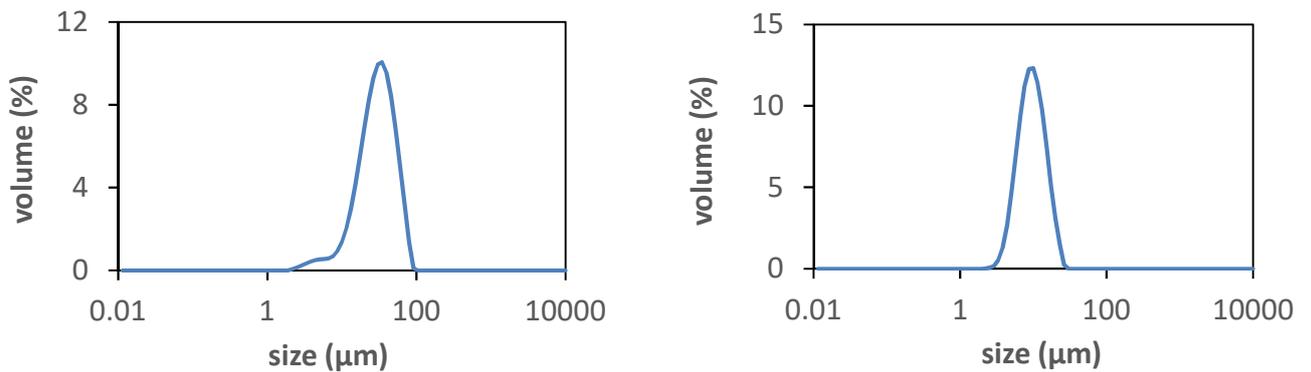


Figure 4: a) Observation by optical microscopy of two reverse emulsions obtained with 10 wt% of PGPR after shearing at 525 s^{-1} (left) and 3150 s^{-1} (right) and b) corresponding drop size distributions measured by static light scattering. The volume-average diameter and polydispersity are $D[4,3] = 30.7 \mu\text{m}$ and $U = 43.9\%$ (left) and $D[4,3] = 9.5 \mu\text{m}$ and $U = 35.5\%$ (right)

Once prepared, to further break the globules, a shear rate larger than the one used for the emulsion preparation is required. This means that an emulsion prepared at a high shear rate is

less sensitive to stirring during use than an equivalent emulsion obtained with an initially lower shear rate. For this reason, we chose the 15 μm -sized reverse emulsion obtained at 1560 s^{-1} with PGPR for the following.

3.3 Formulation of the double emulsion

After this first step, that allowed determining adequate surfactants, two possible couples for the formulation of the double emulsion were selected: PGPR with Tween 20 and PGPR with Tergitol 15-S-12 and two fragrances. For clarity reasons, the systems will be referred as HS/LS/fragrance.

3.3.1 Study of the Tween 20/PGPR/fragrance A or fragrance B system

To vary the HS surfactant concentrations in the intermediate aqueous phase as well as the amount of fragrance, beginning with a direct emulsion composed of 90 wt% of fragrance and 10 wt% to 30 wt% of HS with respect to the aqueous phase, dilutions have been applied. The dilution can be done either with pure water or with an aqueous phase containing the same HS. This direct emulsion was then dispersed in the outer IPM phase containing the LS. Due to the numerous experiments to carry out and time consumed by using the Couette cell, the second emulsification, that is to say the incorporation of the direct emulsion into the IPM, was manually operated (the second shearing with the Couette cell has been skipped). Moreover, the obtained sizes were adequate and a better monodispersity was not targeted. In addition, for application reducing the number of stages is always beneficial. Once obtained, the double emulsions were observed by means of an optical microscope. Several morphologies could be seen. Either the obtained emulsion was indeed double or it turned out to be simple. In many cases, observation was not sharply defined, a coexistence between double or simple drops was seen. Then, the dominant behavior was reported. In some other cases, no emulsion at all could be obtained. All

the results are reported in Figure 5 in the form of diagrams. Despite the fact that these observations are qualitative, they are reproducible.

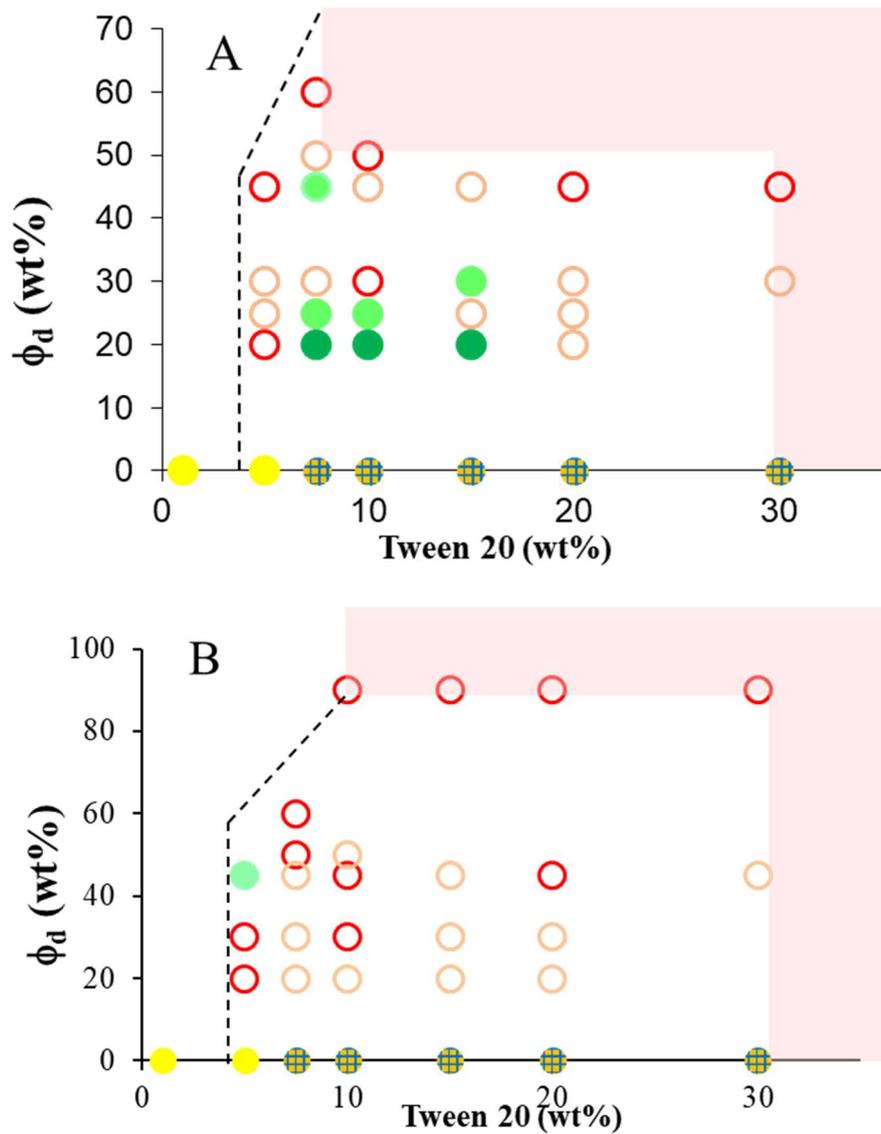


Figure 5: Feasibility diagrams of double emulsions stabilized by 10 wt% of PGPR and various amounts of Tween 20 (x-axis). The inner fragrance droplet volume fraction in the globules ϕ_d has also been varied. Top for fragrance A and bottom for fragrance B.

○ Very few multiple droplets,
 ○ Few multiple droplets in coexistence with simple ones,
 ● Multiple droplets with few simple ones,
 ● Multiple emulsions,
 Multiple droplets (without fragrance),
 ● Reverse emulsion
 - - - Limit of dilution for the direct emulsion
 Area not accessible for this system of double emulsion.

The limit of dilution of the direct emulsion (— —) represents the limit of accessible HS concentration that depends on the minimal concentration possible for the stabilization of the direct concentrated emulsion. Indeed, in the case of Tween 20/PGPR/Fragrance B, the direct emulsion could be prepared with HS concentration from 10 wt% to 30 wt%. As an example, to obtain a system at $\phi_d = 30$ wt% and %HS = 7.5 wt%, a direct emulsion at 90 wt% of droplets stabilized by 20 wt% of Tween20 was diluted three times with water containing 0.83 wt% of HS.

The line at 0 wt% of droplets fraction (x-axis) corresponds to emulsions prepared with an aqueous phase (0.1 M NaCl) containing different concentrations of Tween 20 dispersed and an oil phase composed of IPM and 10% of PGPR. This line without fragrance ($\phi_d=0$) allows evidencing interaction between the two surfactants. Indeed, two different behaviors can be observed. Until 5 wt% of Tween 20, the obtained emulsions are simple reverse emulsions, showing that PGPR dominates the emulsion type. Above 5 wt% of Tween 20, multiple emulsions can be observed (see the micrographs in Supporting information S5 for 1 and 10 wt% of Tween 20), showing the competition of the two surfactants.

Several behaviors are visible on the “diagrams”. It is worth noticing that we could not find any composition where only double globules could be observed, the samples were always “polluted” by the coexistence with empty globules. Moreover, only small areas of compositions allowed obtaining multiple emulsions (Figure 6) and the domains even almost vanished for fragrance B.

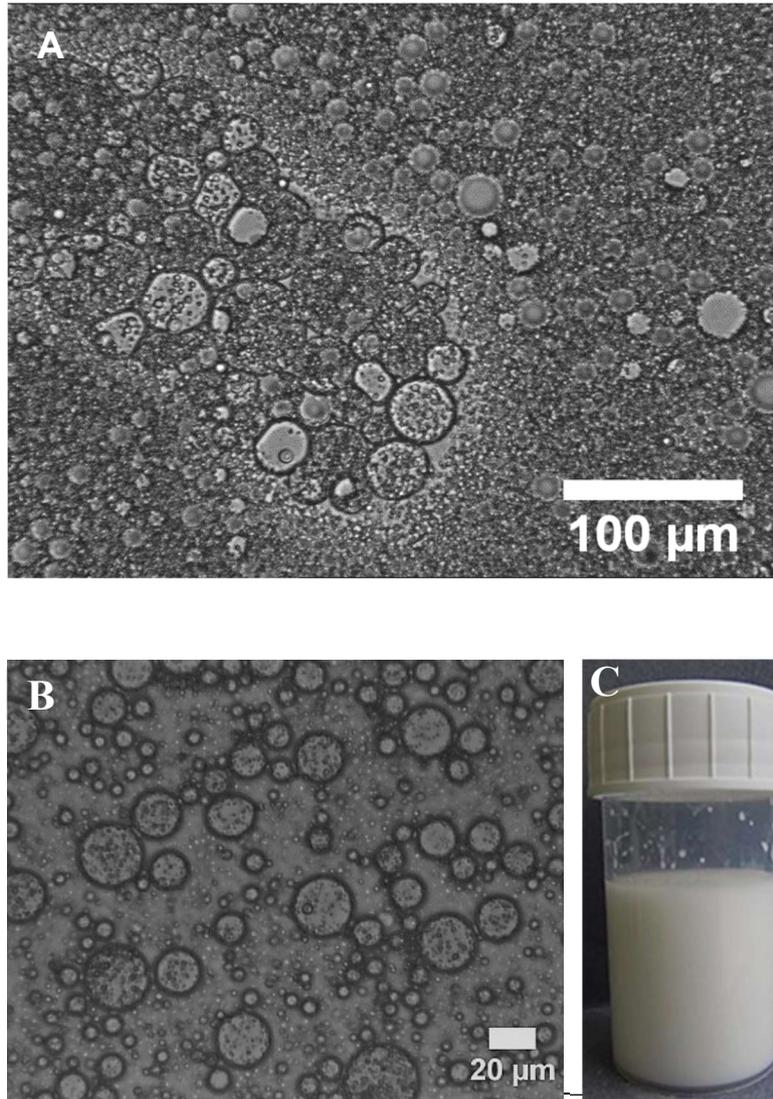


Figure 6: Multiple emulsions for A) fragrance A with $\phi_d=20$ wt% and 10 wt% of Tween 20, B) fragrance B with $\phi_d=45$ wt% and 5 wt% of Tween 20 and C) aspect of the double emulsion corresponding to the micrograph B.

The main area of the diagrams corresponds to compositions where multiple globules coexist with a high number of simple globules or where only simple globules are visible. The fact that there exist no common zone where only double emulsions could be observed, that is to say globules filled with fragrance droplets, indicate that the mixture of surfactant PGPR/Tween 20 is not efficient in stabilizing direct and reverse emulsions. Indeed, coexistence has already been

observed in zones of formulation-composition maps where there is a conflict on the type of emulsions [48-50].

In order to get better insight into the reason of such a difficulty to get double emulsions, we measured the interfacial tension between the aqueous phase containing various concentrations of Tween 20 and the oil phase: IPM containing PGPR (Figure 7) without fragrance (corresponding to the x-axis of Figure 5).

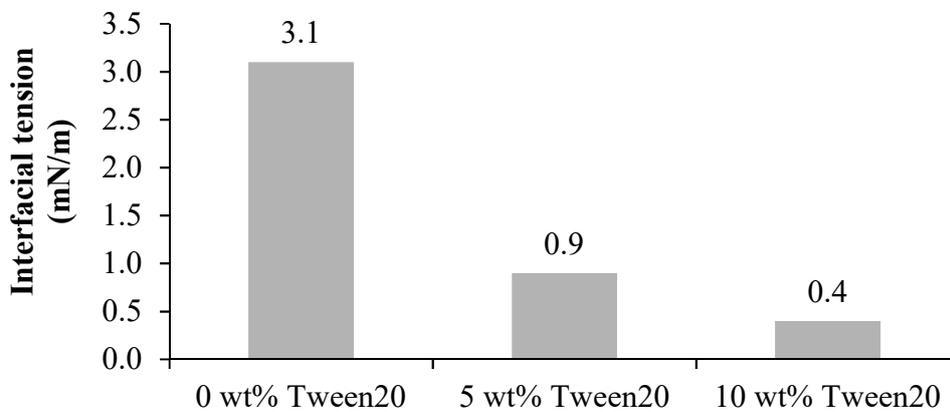


Figure 7: Evolution of the interfacial tension between the aqueous phase containing Tween 20 or not and the IMP oil phase containing PGPR.

A sharp decrease of interfacial tension can be observed in presence of Tween 20. Such a high interaction between the two surfactants likely explains the difficulty to stabilize multiple emulsions. Moreover it can be noticed that the resulting interfacial tensions are very low in presence of Tween 20 (<1 mN/m). This very low interfacial tension favors the formation of an IPM-in water-in IPM emulsion (inner and outer oils are identical) in a one-step process. However, we think that a low interfacial tension is not appropriate when the two oils are different, that is to say when $\phi_d > 0$. Indeed, it is our understanding that the direct fragrance-in-water emulsion should be resistant during its incorporation into the IPM oil. It is known, in

enhanced oil recovery, that low interfacial values are detrimental for the stability of emulsions and systems leading to very low interfacial tension were sought after for this reason [51-55].

3.3.2. Study of the PGPR/Tergitol 15-S-12/Fragrance A or B system

The other hydrophilic surfactant still under consideration was Tergitol 15-S-12, with the same lipophilic surfactant (PGPR at 10 wt% with respect to IPM oil). Again, “feasibility diagrams” have been established. They are reported on Figure 8.

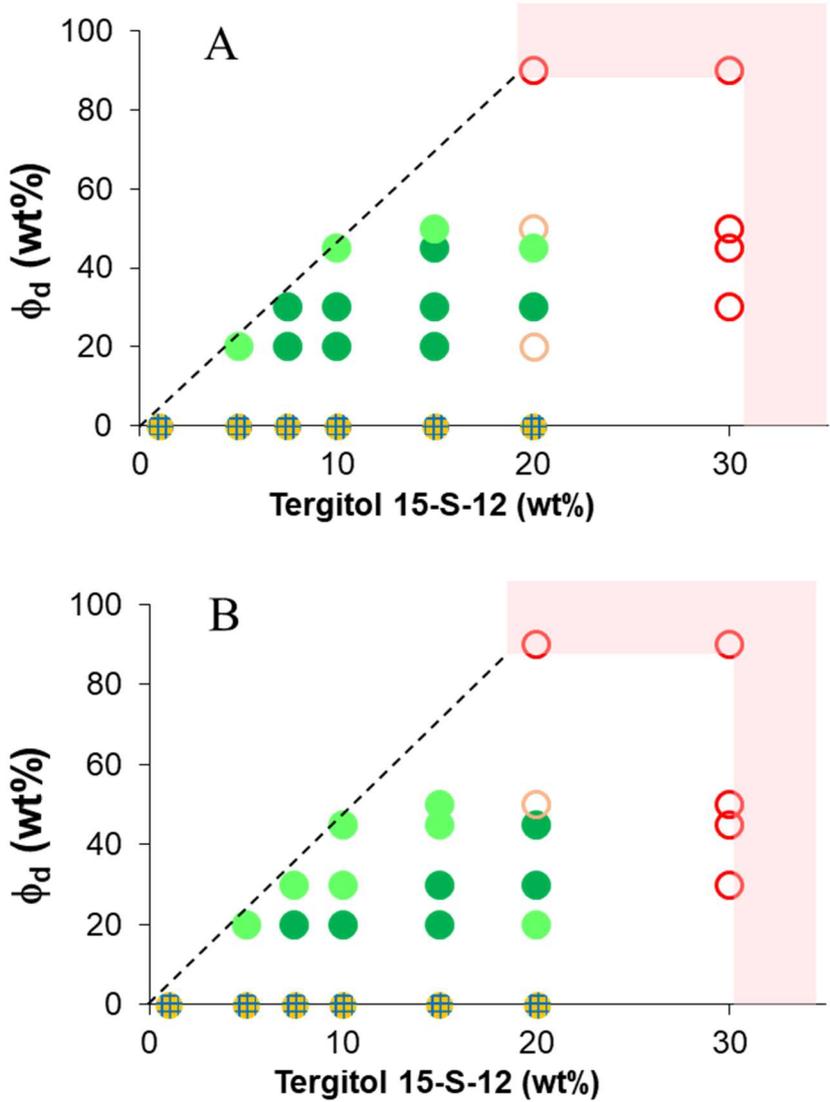


Figure 8: Feasibility diagrams of double emulsions stabilized by 10wt% of PGPR and various amount of both Tergitol 15-S-12 and inner fragrance droplet volume fraction in the globules ϕ_d . Top for fragrance A and bottom for fragrance B. ○ Very few multiple droplets ○ Few multiple

droplets in coexistence with simple one ● Only multiple droplets ● Multiple droplets with few simple one 🌐 Multiple droplets (without fragrance) - - - Limit of dilution for the direct emulsion 🟫 Area not accessible for our system of double emulsion.

Compared to the system with Tween 20, less compositions are accessible by dilution because the minimal concentration needed to stabilize the direct emulsion was higher. Thus, the dilution maximum leads to higher HS surfactant. However, more compositions allow obtaining only or a majority of multiple globules (dark and light green symbols respectively in Figure 8). It is also noticeable that the diagrams with both fragrances look very similar. It seems that Tergitol 15-S-12 is less sensitive to the fragrance. From the diagrams, it can also be concluded that theoretical encapsulations from 15 wt% ($\phi_g = 20\%$) to about 34 wt% ($\phi_g = 45\text{wt}\%$) of fragrance are accessible.

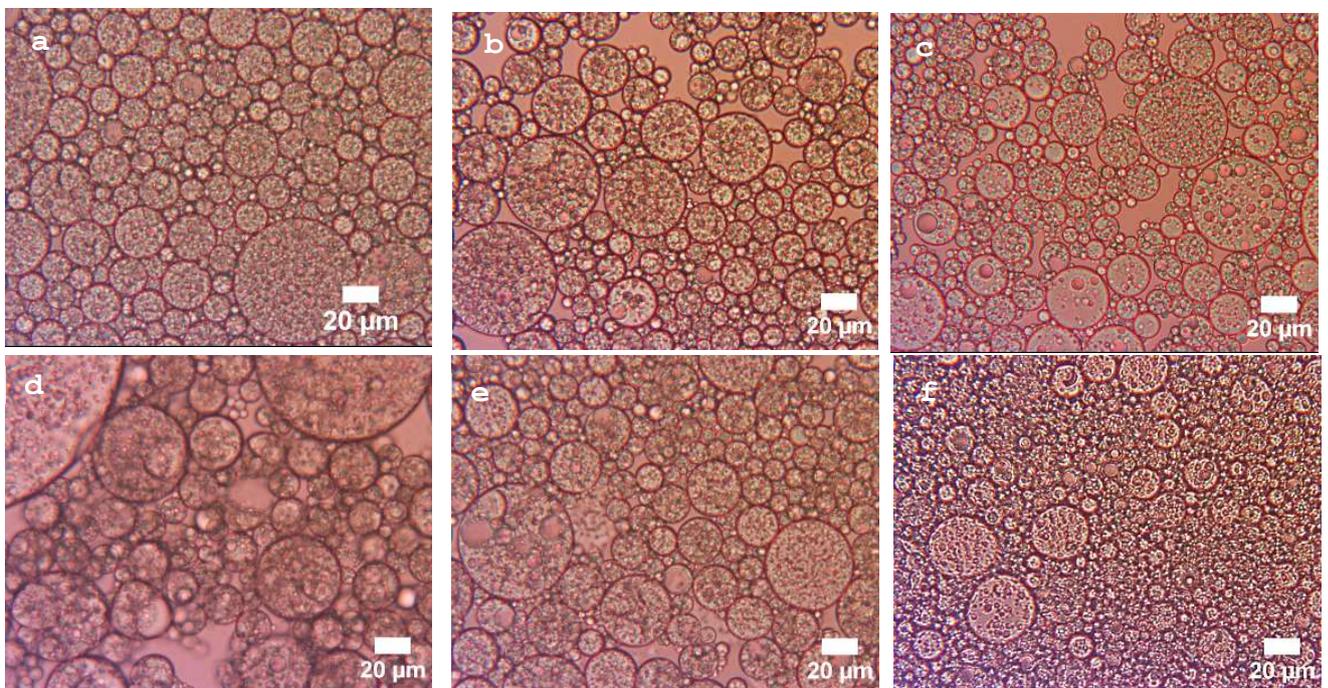


Figure 9: Optical microscopy pictures of the different double emulsions with fragrance A (top a to c) and B (bottom d to f). In all cases, PGPR=10 wt%, globule fraction 75 wt% various amounts of fragrance ϕ_d and Tergitol 15-S-12 (a) $\phi_d = 20$ wt% and HS=15 wt%, (b) $\phi_d = 30$

wt% and HS = 10 wt%, (c) $\phi_d = 30$ wt% and HS = 15 wt%, (d) $\phi_d = 20$ wt% and HS = 7.5 wt%, (e) $\phi_d = 30$ wt% and HS = 15 wt%, (f) $\phi_d = 45$ wt% and HS = 20 wt%.

Some rules can be deduced from these diagrams. The higher the droplet fraction is, the more multiple globules are observed up to a limiting concentration of 50 wt%. Also, the lower the amount of hydrophilic surfactant in the aqueous phase is, the larger proportion of multiple globules (globules containing small droplets) are observed. Thus, a balance has to be found in terms of homogeneous filling and globule size, and the best composition we have identified is 20 wt% of fragrance droplets and 7.5 wt% of Tergitol 15-S-2. In order to test the resistance of this formula with respect to the fragrance variability, a third fragrance C has been tested, and identically a double emulsion could be obtained (see supporting Information S6). The further goal was to polymerize the intermediate aqueous phase. This requires the introduction of monomers into the aqueous phase. Again, the formulation was very resistant to addition of various monomers, cross-linkers and initiators, showing that the proposed formulation is not specific and therefore is of special interest [34].

As polymerization of the aqueous intermediate phase is targeted, the time stability of the double emulsions is not an issue, only a few hours stability was required. Nevertheless, the kinetic stability of some emulsions containing fragrance B was studied over a period of several days. The emulsions were observed by optical microscopy after several days and micrographs are reported in Figure 10 and compared with the initial emulsion (Figure 9) to determine whether destabilization occurred.

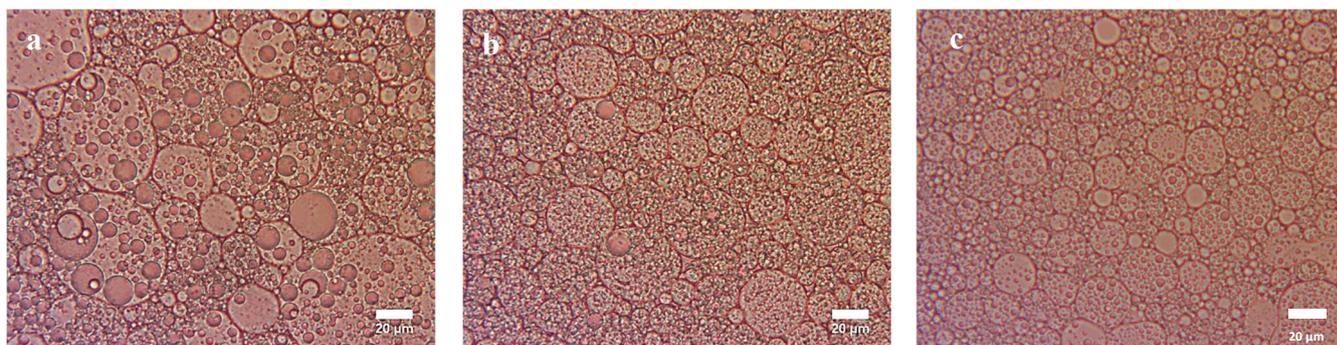


Figure 10: Observation by optical microscopy of the different systems at different times (a) $\phi_d = 20$ wt% and %HS = 7.5 wt% after 3 days, (b) $\phi_d = 30$ wt% and HS = 15 wt% after 4 days and (c) $\phi_d = 45$ wt% and HS = 20 wt% after 10 days.

From the comparison between Figure 9 and Figure 10, different observations can be made. First, all the emulsions remained double and are still composed of multiple globules only, so that no catastrophic and massive destabilization occurred over this period. It is worth noticing that we did not observe any macroscopic phase separation over a period larger than two weeks. Secondly, the emulsion with $\phi_d = 20$ wt% 7.5wt% has evolved in 3 days while the other emulsions are more identical to the initial ones. Globules contained less droplets but they are larger (Figure 10 a). This means that coalescence between inner droplets occurred at this time scale, a phenomenon already described by Pays *et al.* [56] and coalescence between globules also occurred. Note that these phenomena do not change the encapsulation rate it only modifies the size of the compartments. It can also be seen that massive coalescence between droplets and globule, a mechanism described earlier [18,56-58] and detrimental for fragrance encapsulation, neither occurred. As a conclusion, despite some coalescence events, the multiple emulsions remained stable for at least 3 days. This delay was sufficient in the present case, as our goal was to formulate double emulsions, a first step before incorporating monomers and polymerizing the intermediate phase [34]. Note that the stability of double emulsion against encapsulation may vary over a very large range of time depending on the used stabilizers.

Kinetic stability is increased by replacing at least one of the surfactant by a protein, a polysaccharide, fat crystals, particles... that do not desorb and exchange as easily as surfactants. In the present paper kinetic stability was not a goal as double emulsion were thought as an intermediate towards capsules.

3.4 Encapsulation efficiency

The encapsulation rate ϵ of the double emulsion composed of 75 wt% of globules in IPM containing 10 wt% of PGPR with respect to the oil phase and 20 wt% of fragrance B in the globules and 7.5 wt% of Tergitol 15-S-2 with respect to the intermediate aqueous phase has been measured by GC-FID chromatography, immediately after preparation. Possibly the capsules can contain up to 15 wt% of fragrance with respect to the total sample. The GC results showed that the IPM phase diluted at 5% by weight in ethanol contains 0.0315% of fragrance (see Table 6). It means 0.63% of fragrance in IPM phase. $0.63 \times 0,25 / 0,15 = 1.05\%$ of the fragrance was not encapsulated in the double emulsion but was solubilized in the outer IPM phase. In other words, the initial encapsulation rate is as high as 98.95%. GC experiments allow a deeper analysis of the fragrance composition (Table 6). The proportion of each molecule composing the fragrance contained in the IPM phase is given in the eighth column and can be compared to the proportion of the initial fragrance, reminded in the seventh column. Molecules with the largest deviation are noted in red. No clear correlation can be evidenced between these molecules and their hydrophilicity/lipophilicity through its Log P value. Only 1.05% of the initial fragrance amount has not been encapsulated and is solubilized in the outer IPM. Assuming the amount preservation of each molecule the composition of the encapsulated fragrance can be deduced (last column) and compared to the initial fragrance. It can be observed that there is no detectable difference between the initial fragrance and the encapsulated fragrance (comparison between the seventh and ninth column).

Table 6: Dosage of the non-encapsulated fragrance molecules from fragrance B in the continuous IPM phase (dilution at 5%w/w in ethanol): main peaks detected by GC-MS

Retention time (min)	Attribution	Isomer	Proportion of each peak area in the case of the isomers in the fragrance (%)	Proportion of each peak area in the case of the isomers in the sample (%)	Concentration of the fragrance molecule(s) in the diluted sample (10 ⁻³ %)	Proportion of the molecule in the original fragrance (wt%)	Proportion of the molecule in the fragrance in IPM (wt%)	Proportion of the molecule in the encapsulated fragrance (wt%)
7.23	cis-3-Hexenyl acetate	/	/	/	1.1	5	3.5	5.0
8.44	2,4-dimethylcyclohex-3-ene-1-carbaldehyde	(E)-isomer	51	51	2.3	5	7.3	5.0
8.88		(Z)-isomer	49	49				
9.98	prop-2-enyl heptanoate	/	/	/	1.2	5	3.8	5.0
10.71	3,7-dimethyloct-6-en-1-ol	/	/	/	2.9	5	9.3	5.0
10.91	3,7-dimethyl-2,6-Octadienal	(Z)-isomer: neral	34	68	2.1	5	6.7	5.0
11.33		(E)-isomer: geranial	66	32				
11.71	(2-tert-butylcyclohexyl) acetate + 10-undecenal ^a	Cis-isomer	88	49	11.2	43	35.6	43.1
11.99	(2-tert-butylcyclohexyl) acetate	Trans-isomer	12	51				
12.58	2-methoxy-4-(2-propenyl)-phenol	/	/	/	2	5	6.3	5.0

12.82	1-(2,6,6-trimethyl-1-cyclohex-3-enyl)but-2-en-1-one	/	/	/	0,5	2	1.5	2.00
13.45	Tricyclododecanyl Acetate	/	/	/	3.4	10	10.7	10.0
14.19	Methyl ionone (mixture of isomers)	(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one (Methyl ionone Gamma)	63	23	1.2	5	3.8	5.0
14.45		(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one (Methyl ionone delta)	6	23				
14.66		(1E)-1-(2,6,6-trimethylcyclohex-2-en-1-yl)pent-1-en-3-one (Methyl ionone alpha)	21	30				
15.37		(1E)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)pent-1-en-3-one (Methyl ionone beta)	10	24				
14.25	4-(2,6,6-trimethyl-1-cyclohexene-1-yl)-3-butene-2-one	/	/	/	1.2	5	3.8	5.0
17.56	2-Benzylideneoctanal	/	/	/	2.4	5	7.8	5.0
Total					31.5	100	100.1	100.1

^aA coelution is observed between one peak corresponding to (2-tert-butylcyclohexyl) acetate (isomer cis) and the peak corresponding to 10-undecenal

4. Conclusion

The aim of this paper was to determine a unique formulation of fragrance-in-water-in-oil double emulsion able to encapsulate large amounts of fragrance and that resists to fragrance modifications so that all the formulation work has not to be started again when considering another fragrance. The considered model fragrances were composed of a large number of molecules representative of the ones usually employed in industry. As the fragrance is usually lipophilic and the emulsion has to be dispersible in a perfumery solvent, the strategy is to formulate O/W/O emulsions that are not much described in literature (especially in domains others than food). The role of the intermediate aqueous phase is to create a barrier for the diffusion of fragrance. To obtain such double emulsions, two antagonist surfactants have to be chosen: the hydrophilic one for the formulation of the direct (fragrance-in-water) emulsion and the lipophilic one for the stabilization of the globules of inverted emulsion. After sweeping various non ionic stabilizers, PGPR has been selected for the reverse emulsion and Tergitol 15-S-2 has been selected for the direct emulsions. By varying the composition, we could draw “feasibility diagrams” useful to select the optimal composition: 75wt% of globules in IPM containing 10wt% of PGPR with respect to the oil phase, 20wt% of fragrance in the globules and 7.5wt% of Tergitol 15-S-2 in water. This optimal composition is summed up in Table 7 for an easier overview.

Table 7: Composition of the double emulsion valid for three different fragrances. The amounts are given for 100 g of double emulsion

Innermost phase	Intermediate phase	Outermost (continuous) phase
15 g of fragrance	55.5 g of brine 0.1M NaCl 4.5 g Tergitol 15-S-2	22.5 g IPM 2.5 g PGPR
	} 60 g	} 25 g

This composition is adequate for three different fragrances made of numerous molecules representative in perfumery industry, showing its promising use. Such a double emulsion allows encapsulating in theory 15 wt% of fragrance with respect to the total sample. The encapsulation yield is as high as 98.95% of the fragrance that is not altered by the emulsification process. These double emulsions are kinetically stable for at least 3 days, making these systems very interesting by themselves or for more advanced capsules as for example capsules that could be obtained by polymerizing the intermediate aqueous phase.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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